

# Bioinformatic approaches to regulatory genomics and epigenomics

376-1347-00L - 2023 | week 11

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# Debriefing on the assignments

```
job <- submitGreatJob(gr=split_regions["3"], bg=regions, species="hg38")
```

Background matters and should always be stated!

# Today: DNA Methylation

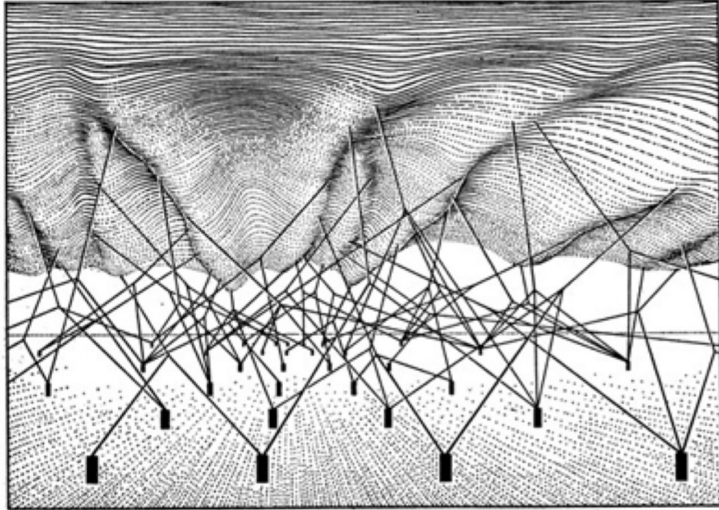


FIGURE 5

*The complex system of interactions underlying the epigenetic landscape. The pegs in the ground represent genes; the strings leading from them the chemical tendencies which the genes produce. The modelling of the epigenetic landscape, which slopes down from above one's head towards the distance, is controlled by the pull of these numerous guy-ropes which are ultimately anchored to the genes.*

Waddington, 1957

so far we looked at:

- histone marks (chIP-seq)
  - H3K27 acetylation
  - H3K27 methylation
  - H3K9 methylation
  - etc.
- chromatin accessibility (ATAC-seq)

# Today: DNA Methylation

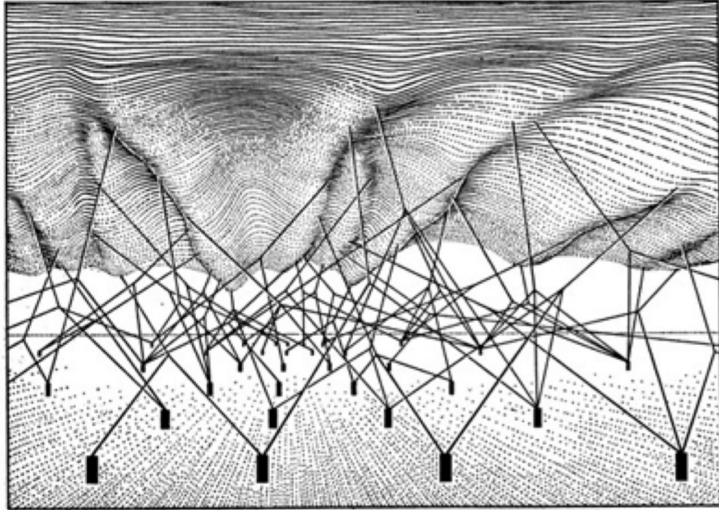


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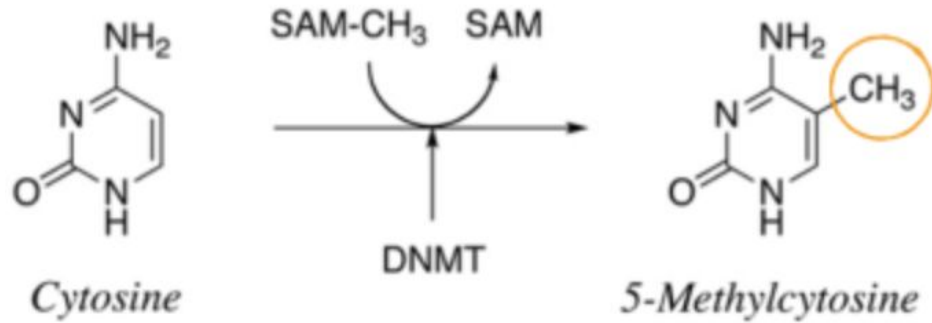
=> Missing piece: DNA methylation

# Plan for Today

- Biology of DNA Methylation:
  - Contexts
  - Activation vs repression
- Profiling Techniques
- Practical
  - visualization
  - bsseq objects
  - enrichment

# DNA Methylation context

CpG Dinucleotides:

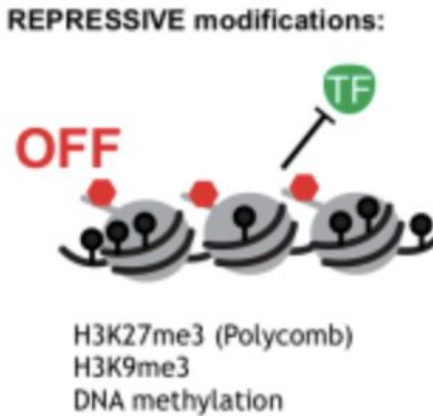
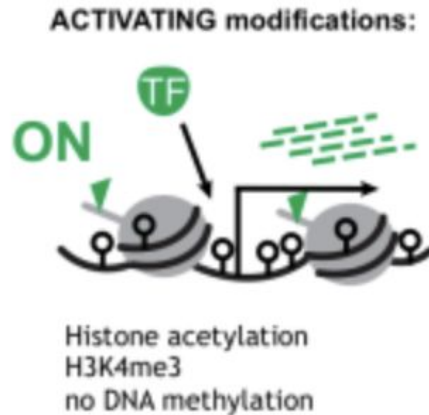


Scheme from Izaskun  
Mallona/Tuncay Baubec

also in other contexts such as CHG, CHH...

# DNA Methylation: Activation vs Repression

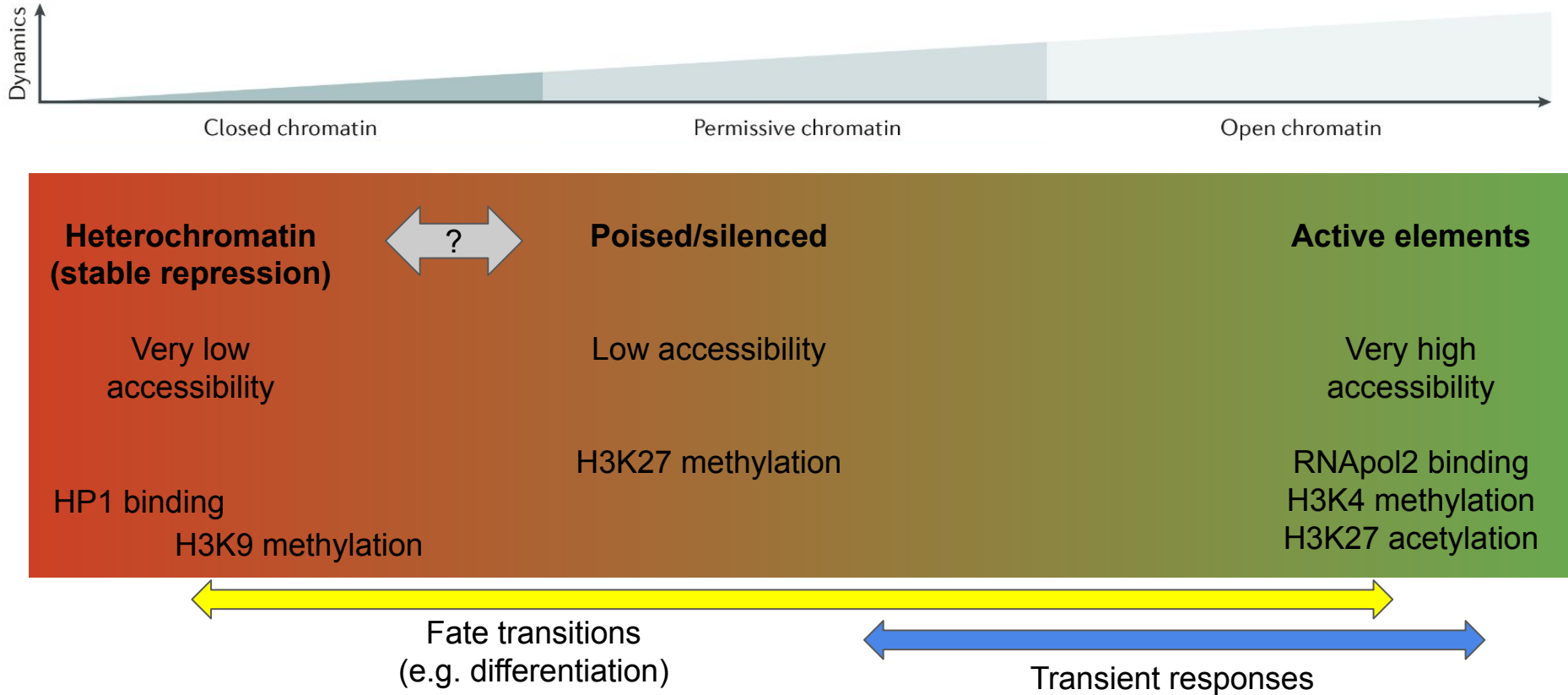
Repressive role of DNA methylation in transcription



Scheme from Izaskun  
Mallona/Tuncay Baubec

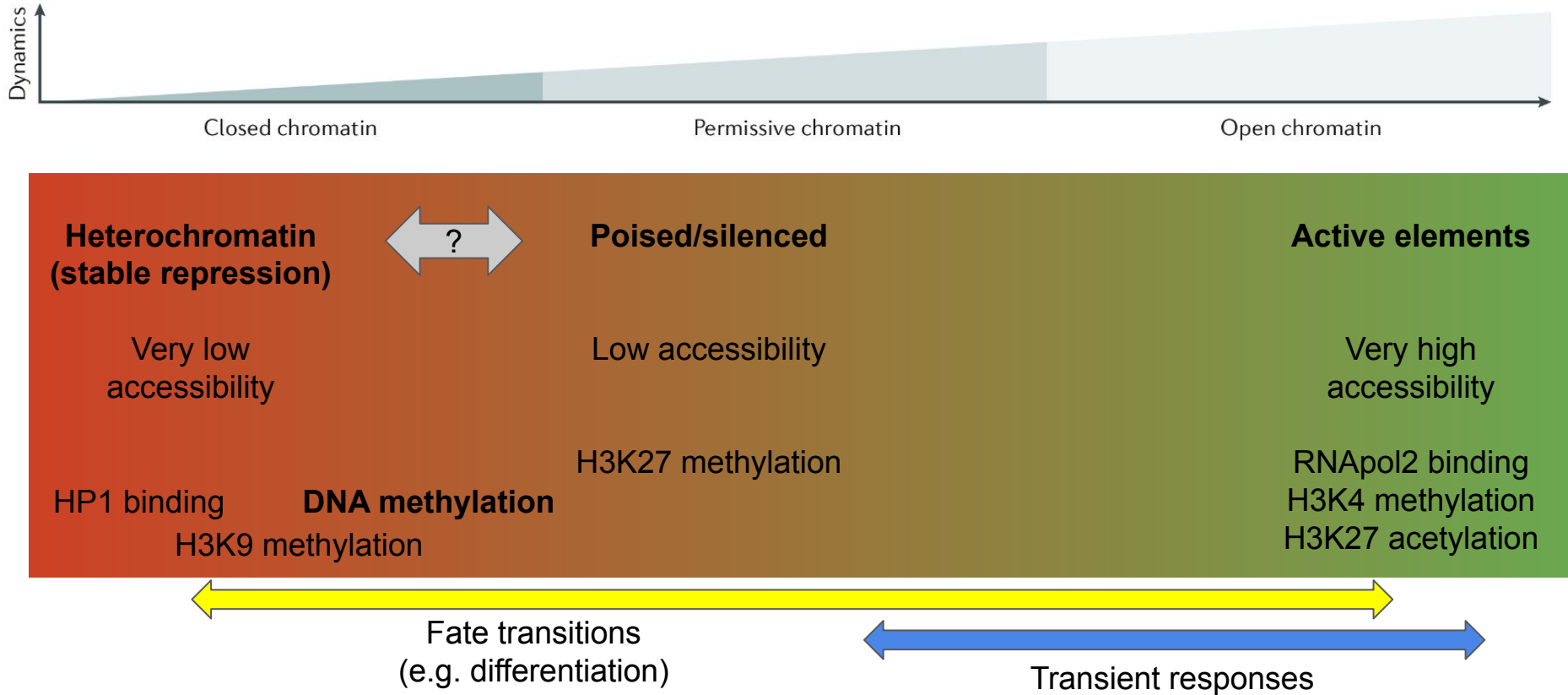
Correlation between DNA methylation and gene silencing

# There are degrees of accessibility (activation/repression)

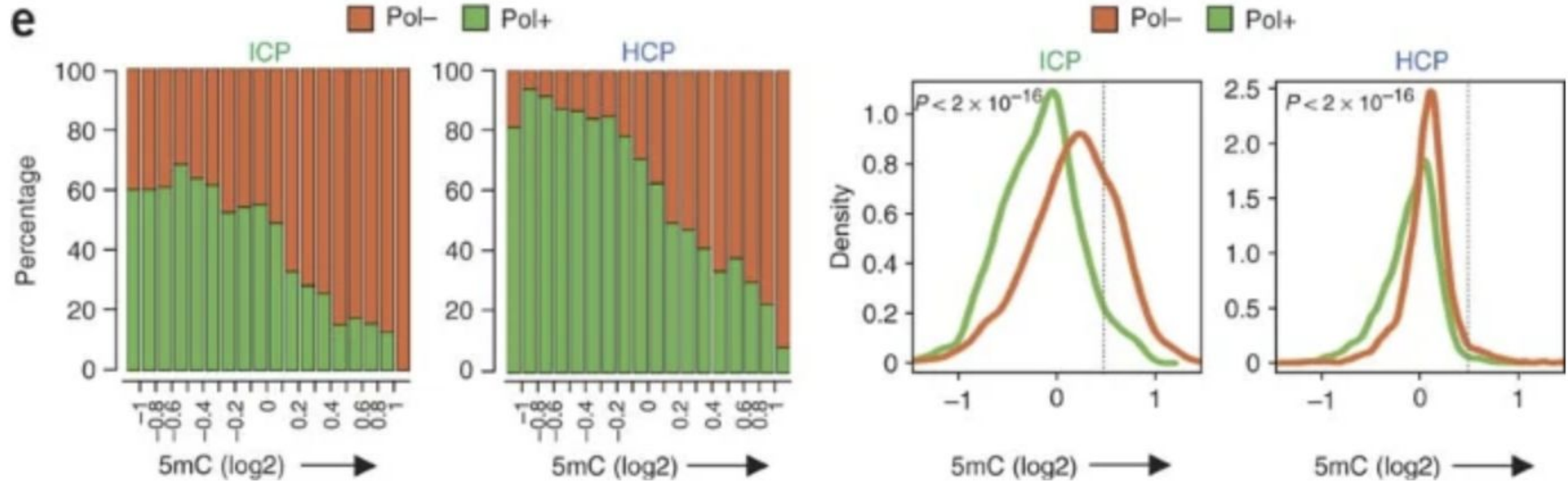




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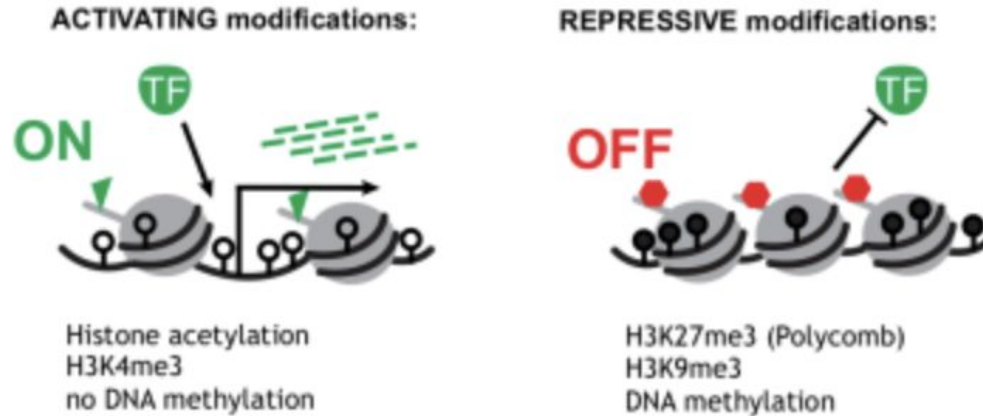
at promoters



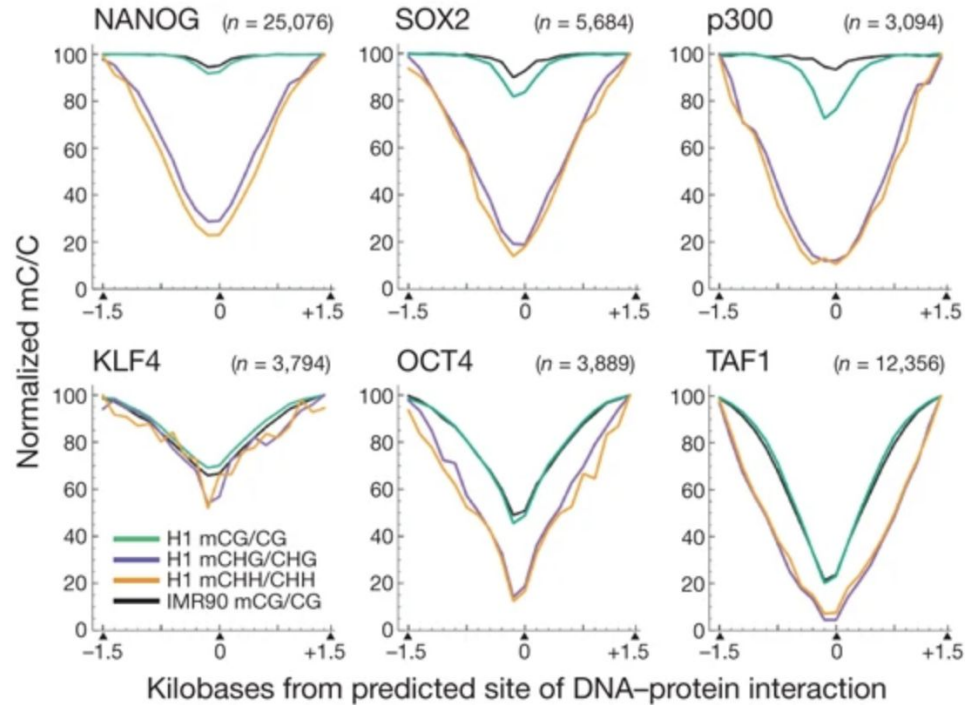
Weber et al., 2007

## at promoters

*“However, how this leads to transcription inhibition is still not entirely resolved, as the methyl mark per se does not seem to confer silencing. Regions of accessible chromatin are frequently lowly methylated or unmethylated, indicating that binding of transcription factors and DNA methylation are mutually exclusive”. (Greenberg et al. 2019)*

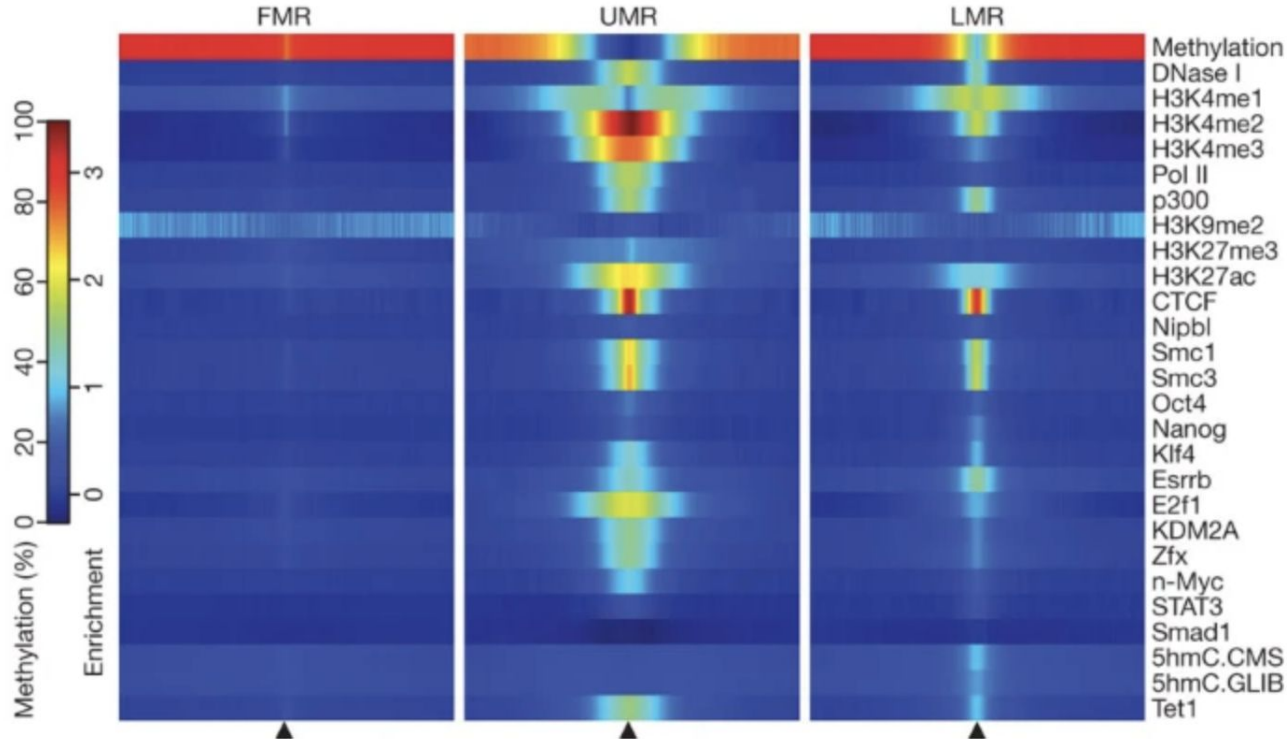


# TF Binding & Methylation



Lister et al., 2009

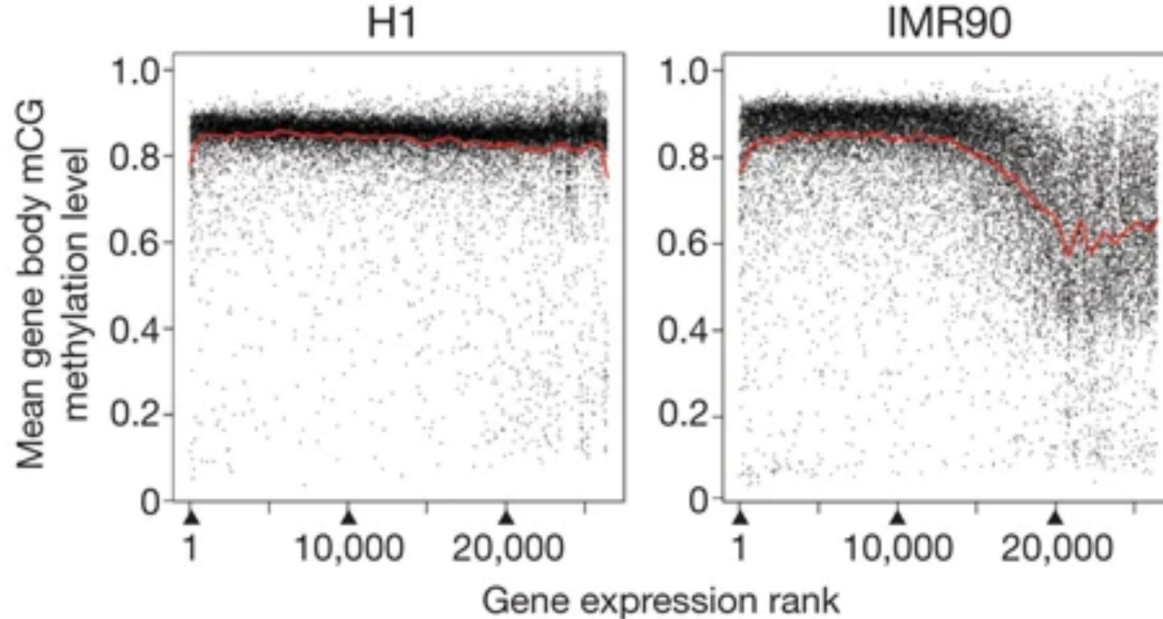
# TF Binding & Methylation



Stadler et al., 2011

# at gene bodies

*“The enrichment of DNA methylation in gene bodies presents a paradox: on the one hand, gene-body methylation is highly conserved across eukaryotes — more than it is conserved at transposable elements, for example — indicating it has an important function. On the other hand, DNA methylation is mutagenic, so why is it so prominent in coding sequences” (Greenberg et al. 2019)*



Lister et al., 2009

# Practical - Part 1

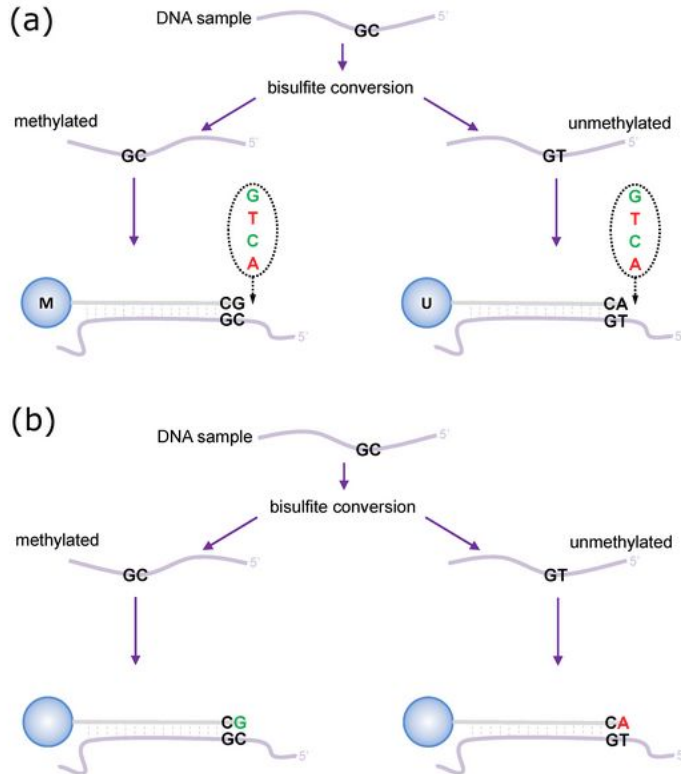
Visualization

# Profiling techniques

- DNA Methylation Arrays:
  - Illumina 450K/850K
- Bisulfite sequencing:
  - RRBS
  - WGBS
  - scWGBS



# Methylation Arrays



- fluorescence signal
- cheap(er)
- again Illumina:
  - Infinium 450K & 850K probing wide ranges of the genome
  - two types of assays: Infinium I (a), Infinium II (b)
  - divergence between the  $\beta$ -values retrieved from the type I and type II Infinium assays. Specifically, the  $\beta$ -values obtained from Infinium II probes were less accurate and reproducible than those obtained from Infinium I probes. (Dedeurwaerder et al., 2011)
- Analysis:
  - [A cross-package Bioconductor workflow for analysing methylation array data.](#)

# Beta & M-Values

- Beta-value, ratio of the methylated probe intensity and the overall intensity:

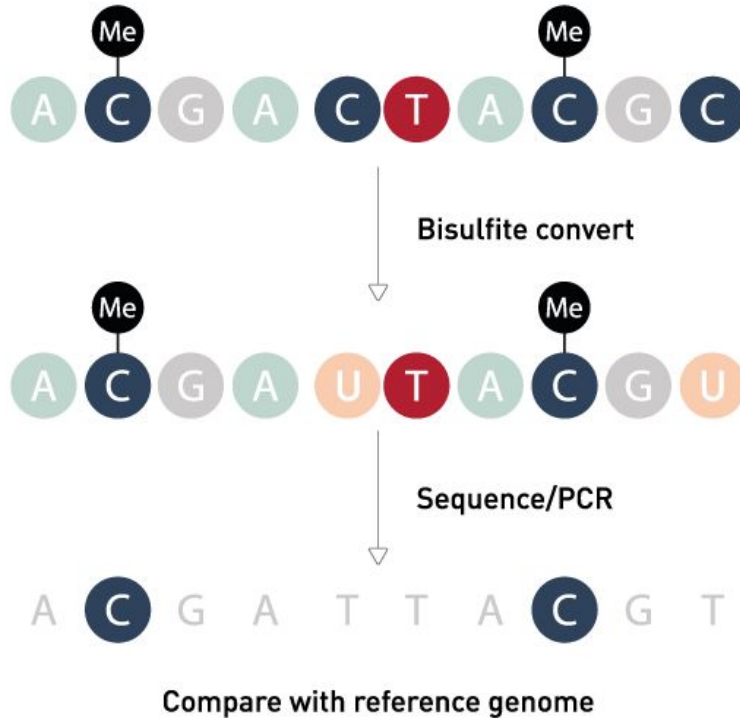
$$Beta_i = \frac{\max(y_{i,methy}, 0)}{\max(y_{i,unmethy}, 0) + \max(y_{i,methy}, 0) + \alpha}$$

- M-value, log2 ratio of the intensities of methylated probe versus unmethylated probe:

$$M_i = \log_2 \left( \frac{\max(y_{i,methy}, 0) + \alpha}{\max(y_{i,unmethy}, 0) + \alpha} \right)$$

- The Beta-value has a more intuitive biological interpretation, but the M-value is more statistically valid for the differential analysis of methylation levels. (Du et al., 2010)

# Bisulfite Sequencing



Exists in different flavors:

- RRBS
- WGBS
- scWGBS

# Bisulfite Sequencing - Aligning

## Bismark



CpG-report file:

	chr	pos	strand	met	unmet	context	tri-context
1:	5	11823	+	0	0	CG	CGA
2:	5	11824	-	0	0	CG	CGG
3:	5	11850	+	0	0	CG	CGA
4:	5	11851	-	0	0	CG	CGG
5:	5	11856	+	0	0	CG	CGA

In R: bsseq package:

```
read.bismark()
```

# Bisulfite Sequencing - Aligning

## Bismark



CpG-report file:

	chr	pos	strand	met	unmet	c	
1:	5	11823	+	0	0		
2:	5	11824	-	0	0		CG
3:	5	11850	+	0	0		CGG
4:	5	11851	-	0	0		CGA
5:	5	11856	+	0	0		CGG

met	unmet
0	0
20	20
1	2
10	20

In R: bsseq package:

```
read.bismark()
```

# DNA-Methylation Analysis tasks:

- Smoothing:
  - [bsseq](#)
- Differential Methylation testing:
  - [DMRcate](#) (defines DM regions itself)
  - [edgeR](#) (allows for testing user-defined regions, e.g. genes of interest)
  - Many more (Methylkit, bsseq etc.)
- Calling partially methylated domains (PMDs)
  - [MethylSeekR](#)
- Quantifying DNA methylation heterogeneity
  - Quantitative comparison of within-sample heterogeneity scores for DNA methylation (Scherer et al., 2020)

# Practical - Part 2

bsseq objects + Differential Methylation Testing

# assignment

1. Plot a heatmap of the methylation levels of the genes in top 5 DMR regions.
2. Run an enrichment analysis (rGREAT) of the genes within DMRs vs all genes of chromosome 22.
  - a. Describe what your enrichment analysis is testing exactly & describe the results.



# References

- Weber, M., Hellmann, I., Stadler, M. et al. Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome. *Nat Genet* 39, 457–466 (2007). <https://doi.org/10.1038/ng1990>
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- Dedeurwaerder S, Defrance M, Calonne E, Denis H, Sotiriou C, Fuks F. Evaluation of the Infinium Methylation 450K technology. *Epigenomics*. 2011 Dec;3(6):771-84. doi: 10.2217/epi.11.105. PMID: 22126295.
- Du, P., Zhang, X., Huang, CC. et al. Comparison of Beta-value and M-value methods for quantifying methylation levels by microarray analysis. *BMC Bioinformatics* **11**, 587 (2010). <https://doi.org/10.1186/1471-2105-11-587>
- Greenberg, M.V.C., Bourc'his, D. The diverse roles of DNA methylation in mammalian development and disease. *Nat Rev Mol Cell Biol* **20**, 590–607 (2019). <https://doi.org/10.1038/s41580-019-0159-6>